To the Editor:

Measurements of urine or plasma metanephrine (MN)\(^1\) and normetanephrine (NMN) are considered pivotal tests in the diagnosis of pheochromocytoma and paraganglioma. There has also been increasing interest in measuring 3-methoxytyramine (3MT), the corresponding O-methylated metabolite of dopamine. 3MT measurement might allow earlier diagnosis of rare cases of pheochromocytoma and paraganglioma that secrete predominantly dopamine (1–3). 3MT measurement might also enable stratification of familial cases by secretion patterns and play a role in predicting the malignant potential of pheochromocytoma and paraganglioma (2).

Eisenhofer and others have used HPLC–electrochemical detection (HPLC-ECD) to measure 3MT in plasma (1–4). de Jong et al. developed an online solid-phase extraction liquid chromatography–tandem mass spectrometry (LC-MS/MS) method that simultaneously measures MN and NMN, but not 3MT, was found to produce a measurable 3MT ion peak, although the ion pairs do not appear related (\(m/z\): 3MT, 151.1/91.1; MN, 180.1/148.1; NMN, 166.1/134.1). The MN contribution to the 3MT peak was nearly 2% of the injected MN concentration. In a patient sample with a relatively high MN concentration, this amount of cross talk will lead to overestimation of the normally much lower endogenous 3MT concentration (Fig. 1, left). Unfortunately, ionic cross talk also impedes the use of the less-intense qualifier ion pair (\(m/z\) 151.1/119.1).

We explored this phenomenon further by performing product ion scans of the 180 \(m/z\) ion during infusion of 10 \(\mu\)g/mL MN in 700 mL/L methanol at a flow rate of 10 \(\mu\)L/min. This experiment revealed the predicted dominant product ions at \(m/z\) 165 and \(m/z\) 148, as well as a product ion of \(m/z\) 151—which corresponded to the 3MT precursor ion—at an approximately 10-fold lower intensity. During this infusion, a product ion scan of \(m/z\) 151 showed a typical 3MT fragment of \(m/z\) 91, and a precursor ion scan of the \(m/z\) 91 ion gave precursors of \(m/z\) 151 and \(m/z\) 180. We also observed a similar fragmentation pattern with the deuterium-labeled MN (MN-d3) internal standard, NMN, and the NMN-d3 internal standard, but to a lesser extent.

This discovery suggested that MN and, to a lesser extent, MN-d3, NMN, and NMN-d3 had the ability to fragment within the source into ions mimicking 3MT. The MN, NMN, and 3MT precursor ions usually represent a hydroxyl loss and the addition of a charged H\(^+\). A proportion of MN, MN-d3, NMN, and NMN-d3 might also lose methyl or hydroxy groups to give 3MT precursor ions. Considering the formulae of MN, NMN, and 3MT and a range of different ionization conditions, variable proportions of MN or NMN (or their internal standards) might be “converted” to a 3MT mimic.

On the basis of these findings, we attempted to optimize source conditions to reduce this cross talk but were unable to strike a balance between reduced cross talk and required analytical sensitivity. Chromatographic separation of MN and 3MT, which was absent in the initial method, proved necessary to resolve this issue. We found that ion-pairing liquid chromatography using 1.25 mmol/L perfluoropentanoic acid as the ion-pairing agent (gradient from 100 to 400 mL/L methanol; flow rate, 0.5 mL/min) and an Agilent Zorbax Eclipse XDB-C18 column (4.6 × 50 mm, 3.5 \(\mu\)m) for separation resolved 3MT from MN effectively, achieving a 60-s separation between the 2 peaks (Fig. 1, right). Not surprisingly, a peak corresponding to the monitored 3MT ion pair could still be seen at the MN retention time, further emphasizing the importance of peak resolution.

Our work suggests that cross talk needs to be addressed and evaluated in any mass spectrometry assay, and if identified, appropriate actions should be taken to minimize cross talk interferences through chromatographic resolution. We found that whenever MN, NMN, and 3MT are not

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\(^1\) Nonstandard abbreviations: MN, metanephrine; NMN, normetanephrine; 3MT, 3-methoxytyramine; HPLC-ECD, HPLC–electrochemical detection; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MN-d3, deuterium-labeled MN.
resolved chromatographically, as in the case of our original method and in the method of de Jong et al., there is the risk of MN or NMN contributing to the 3MT peak. Given that the concentrations of both MN and NMN in plasma are much higher than those of 3MT, that will lead to falsely high 3MT measurements.

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References

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Fig. 1. LC-MS/MS separation of MN and 3MT.
(Left), Original online solid-phase extraction LC-MS/MS method showing contribution from the 5-μg/L (29.9-nmol/L) MN standard (top) to the 3MT ion pair (bottom). 3MT is absent from the injection. (Right), Ion-pairing chromatography showing resolution of MN (top, 1.51 min) and 3MT (bottom, 2.53 min). Note persistence of cross talk in the 3MT ion pair at 1.51 min. Both MN and 3MT were injected.